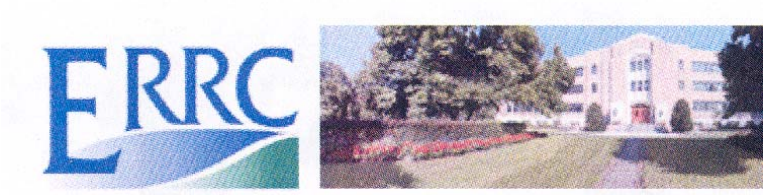

EASTERN REGIONAL RESEARCH CENTER
AGRICULTURAL RESEARCH SERVICE
UNITED STATES DEPARTMENT OF AGRICULTURE
600 E. MERMAID LANE
WYNDMOOR, PA 19038
(215) 233-6400

Title: Improved Antimicrobial Wash Treatments for Decontamination of Apples

Author(s): G.M. Sapers, R.L. Miller, B.A. Annous and A.M. Burke

Citation: Journal of Food Science (2002) 67(5): 1886-1891

Number: 7128

Improved Antimicrobial Wash Treatments for Decontamination of Apples

G.M. SAPERS, R.L. MILLER, B.A. ANNOUS, AND A.M. BURKE

ABSTRACT: We investigated means of improving efficacy of hydrogen peroxide washes in reducing *Escherichia coli* populations on inoculated apples by increasing contact between attached bacteria and the wash solution. Golden Delicious apples were inoculated with *E. coli* and treated with heated 5% H_2O_2 with or without agitation, by spraying and simultaneous brushing or abrading calyx and stem areas, or by vacuum infiltration. Samples were homogenized, diluted, and plated to enumerate surviving bacteria. Population reductions were greater when apples were treated with agitation, by targeted spraying with abrasion, by vacuum infiltration with stem removal, and by application of treatments at 80 °C. However, discoloration occurred at temperatures above 60 °C.

Keywords: apples, decontamination, *Escherichia coli*, hydrogen peroxide, abrasion, infiltration, efficacy

Introduction

INCREASED OCCURRENCE OF PRODUCE-RELATED OUTBREAKS OF FOOD borne illness has focused attention on the efficacy of washing and sanitizing treatments in decontaminating fruits and vegetables (Tauxe and others 1997; NACMCF 1999). Conventional washing and sanitizing agents typically reduce microbial loads by 1 to 2 $\log_{10}$CFU/g (Beuchat 1998; Beuchat and others 1998; Brackett 1999). Studies conducted by the authors with apples, artificially inoculated with a non-pathogenic *Escherichia coli*, have demonstrated somewhat greater population reductions (2 to 3 $\log_{10}$CFU/g) when the apples were washed by immersion in 5% hydrogen peroxide (H_2O_2) at 50 to 60 °C (Sapers and others 1999). However, when this and other experimental and conventional sanitizing treatments were applied with a commercial flat-bed brush washer, population reductions were <1 $\log_{10}$CFU/g (Annous and others 2001). Survival of bacteria during washing was attributed in part to poor contact between apple surfaces and the rotating brushes and sanitizer solutions. Other factors considered to be potentially important in limiting the efficacy of washing were attachment of bacteria in inaccessible sites in the stem and calyx areas or in skin punctures, infiltration of bacteria in the calyx channel, and bacterial incorporation within biofilms (Sapers and others 2000). To achieve greater reductions in microbial populations on produce, improved methods of decontamination that overcome these limiting factors are needed. In the case of apples, such methods might be applied to fruit intended for fresh-cut processing or to apples for fresh market. Our objectives in this study were to determine whether such improvements could be realized by application of H_2O_2 solutions with agitation, with targeted abrasion or brushing of calyx and stem areas, by vacuum infiltration into inaccessible binding sites, or at elevated temperatures.

Materials and Methods

Source and inoculation of apples

Unwaxed Washington State Golden Delicious apples weighing approximately 150 to 180 g were obtained from a single grower and stored at 4 °C until needed for decontamination experiments (usually less than 2 mo). Apples were inoculated with a non-pathogenic *E. coli* strain, ATCC 25922 (except ATCC 35695, in 1 preliminary experiment), used as a surrogate for *E. coli* O157:H7. Inocula were prepared by growing the surrogate in Trypticase Soy Broth (TSB) (Difco, Detroit, Mich., U.S.A.) at 37 °C for 8 h, transferring 100 ml of the late exponential-phase culture to 1 L of TSB, allowing the culture to grow for approximately 18 h at 37 °C, centrifuging the culture at 10,415 × *g* for 10 min, washing once with 200 mL sterile distilled water, and resuspending the cells in 2 L of sterile distilled water for a final cell concentration of approximately 8.8 $\log_{10}$CFU/mL (range of 8.7 to 9.1 $\log_{10}$CFU/mL). Apples, taken from refrigerated storage, were immersed in the stationary phase *E. coli* cell suspension at ambient temperature (approximately 20 °C) for 5 min, drained, placed on their sides in plastic tubs lined with absorbent paper, and held for 24 h at 4 °C prior to use.

Validation of surrogates

Based on the results of surrogate screening studies carried out in our laboratory (Riordan and others 2001), washing trials with representative heated H_2O_2 solutions were carried out to validate the use of 2 potential *E. coli* O157:H7 surrogates for inoculation of apples: *E. coli* ATCC 25922 and 970152. Inocula of these strains and *E. coli* O157:H7 were prepared as described above, and sufficient numbers of apples were inoculated by immersion and refrigerated overnight, as described, so that treatments could be applied to duplicate sets of 4 apples each. Apples were washed in 2L 5% H_2O_2 for 2 min or with 5% H_2O_2 + 1% sodium 2-ethylhexylsulfate (Stepanol, a wetting agent; Stepan Company, Northfield, Ill., U.S.A.) for 1 min, both applied at 60 °C in a 6-L glass beaker placed in a shaker water bath (Innova 3100, New Brunswick Scientific, Edison, N.J., U.S.A.) with agitation at 120 rpm. Samples and untreated controls were each blended and the surviving *E. coli* enumerated on TSA/MAC as described.

Application of antimicrobial treatments

Duplicate or triplicate sets of inoculated apples were treated by immersion in 5% H_2O_2 under various conditions of time, temperature, agitation, application of abrasion, and vacuum, intended to improve contact between the H_2O_2 solution and microbial attachment sites on the apple surface.

Effect of agitation

Apples inoculated with *E. coli* (ATCC 25922) were treated with

5% H_2O_2 at 50 °C or 200 mg/L Cl_2 (total chlorine, calculated from added sodium hypochlorite; adjusted to pH 6.5 with citric acid) at 20 °C for 1 min, with or without agitation. One group of samples was treated by submerging duplicate sets of 3 apples in 2 L of solution in 3-L beakers without agitation. Peroxide solutions were preheated to 50 °C, and the treatments applied by placing the beakers in a 50 °C water bath, adding the apples, and keeping them submerged with a slotted spoon. The same treatments were applied by placing 6 apples in a stainless steel covered basket inserted in a 22-L pail containing 6 L of solution so that the apples would be fully submerged. Agitation was provided by placing the pail on a shaker (G10 Gyrorotary Shaker, New Brunswick Scientific Co., Inc., New Brunswick, N.J., U.S.A.) set to 65 rpm. We refer to this method as a "dynamic wash"; washing with agitation, described elsewhere in this study, was carried out on a shaker (Sapers and others 1999) and was less vigorous than the dynamic washing treatment. The dynamic and static treatments were each carried out in duplicate.

Table 1—Comparison of potential surrogates for *E. coli* O157:H7 in evaluating efficacy of heated H_2O_2 wash treatments for inoculated Golden Delicious apples

	E. coli population reduction[a]	
E. coli strain	5% H_2O_2 @ 60 °C for 2 min	5% H_2O_2 + 1% SHS[b]@ 60 °C for 1 min
ATCC 25922	2.72[c]	2.30[c]
ATCC 970152	1.56[d]	2.32[c]
0157:H7	2.32[c]	2.14[c]

[a]Based on inoculated control populations of 4.87/4.53, 5.12/5.20, and 5.12/5.22 for ATCC 25922, ATCC 970152, and O157:H7, with or without SHS in wash, respectively.
[b]SHS = sodium 2-ethylhexylsulfate.
[c, d]Means of duplicate trials; means within the same column with no letter in common are significantly different at $p < 0.05$ by the LSD test.

Application of abrasion

To improve contact between sanitizing solutions and inaccessible bacteria attached in the stem and calyx areas, 6 apples inoculated with *E. coli* (ATCC 25922) were lightly abraded in these areas with a 19 mm × 22 mm conically shaped aluminum oxide grinding point (Vermont American Tool Company, Lincolnton, N.C., U.S.A.), attached to an electric drill. Prior to abrading, duplicate sets of 3 apples were washed by immersion for 1 min in 2 L 5% H_2O_2 at 50 °C with agitation and then pooled. During application of the abrasion treatment to the individual apples, the abraded surface was flushed to remove debris by applying a stream of de-ionized water with a wash bottle. After abrading, the apples were washed again either in a colander under tap water for 30 s or as sets of 3 in 2 L 5% H_2O_2 at 50 °C, followed by the tap water rinse. Following treatment, the treated apples and 6 inoculated control apples were cored with a sterile, stainless steel cork borer (27 mm dia) to separate the stem, core, and calyx portions from the edible portion. The separated cores were dissected aseptically to isolate stem and calyx portions, which were pooled and weighed, as described (Sapers and others 2000), so that population reductions in the calyx and stem areas could be determined. Additional samples and controls (sets of 6 apples) were evaluated without dissection in duplicate trials so that overall population reductions due to the abrasion treatments could be determined. Weight losses resulting from the abrasion treatment also were determined.

Targeted brushing

Because damaging abrasion treatments would not be suitable for fruit intended for fresh market, the efficacy of brushing calyx and stem areas without using sufficient force to break the skin was examined. In a preliminary experiment, brushing treatments were applied to sets of 6 apples inoculated with *E. coli* ATCC 35695 (K-12) as the surrogate with an electric toothbrush (Oral B Plaque Remover, Model D 6011, Braun, Inc., Woburn, Mass., U.S.A.). Prior to brushing, the apples were washed by immersion of sets of 3 apples for 1 min with agitation in 2 L 5% H_2O_2 + 1% APL Kleen® 245 (an acidic detergent formulation; Elf Atochem North America Inc., Agrichemicals Div., Decco Dept., Monrovia, Calif., U.S.A.) at 50 °C and then rinsed with water. Apple calyx and stem areas were individually brushed for 1 min with the electric toothbrush using a 5% H_2O_2 flush or an abrasive paste prepared from 8 g calcinated oyster shell powder (provided by K. Isshiki, National Food Research Institute, Tsukuba, Japan; see Isshiki and Azuma (1995)) + 8 g H_2O. The paste was applied dropwise to the calyx and stem areas with a transfer pipette until these surfaces were thoroughly coated. After brushing, the apples were rinsed with warm tap water for 15 s. Treated apples and controls were cored and dissected as described above.

To determine the effects of brushing calyx and stem areas with calcinated oyster shell paste on a whole apple basis, we conducted a follow-up series of experiments 2 y after the initial experiments in which the brushing procedure was slightly modified to provide better control, and the surrogate was *E. coli* ATCC 25922. In these trials, a digital force gauge (Model FGE 0.5; Shimpo Instruments, Itasca, Ill., U.S.A.), placed in contact with the upper surface of the electric toothbrush above the rotating brush, was used to measure the force applied to each apple during brushing. Duplicate sets of 3 inoculated apples were washed with 5% H_2O_2 + 1% APL Kleen® 245 with agitation and rinsed with water as described above. Then, individual apples were clamped to a ring stand, oriented with the calyx facing up, and about 0.5 mL of an oyster shell/water paste (1:2) was applied by transfer pipette to the calyx area. Each apple was raised by elevation of a lab jack until contact was made with the rotating brush, and a force reading of 100 g ± 5 g was obtained; the calyx was brushed for 1 min with periodic adjustment of the lab jack, as needed to maintain the desired force. The apple was then lowered, repositioned in the clamp so that the stem faced up, and brushed in the stem area with 0.5 mL calcinated oyster shell paste as described above, but with the stem aligned off-center to avoid interference with the brush rotation. Each apple was rinsed with tap water for 30 s after both calyx and stem areas were brushed to remove residual paste. The pooled sets of 3 apples were homogenized without prior coring and dissection. The calcinated oyster shell abrasion treatment was compared with an inoculated control in duplicate trials, and the experiment was repeated twice.

Vacuum infiltration

Sanitizing solutions were applied under vacuum to improve penetration of the sanitizing agent into stem or calyx areas punctures or pores and increase exposure of bacteria attached in these locations. Twelve sets of 4 apples each were inoculated with *E. coli* (ATCC 25922), and after 24 h at 4 °C, half of the sets were de-stemmed. Sets of 4 inoculated apples were submerged in 2 L preheated 5% H_2O_2 at 45 °C in a 3-L beaker, which was placed in a vacuum oven (Model 3608, Lab-Line Instruments Inc., Melrose Park, Ill., U.S.A.). A vacuum was applied over 3 min, reaching 508 mm Hg at about 1 min, at which point the vacuum line was closed to prevent boiling of the H_2O_2 solution. After treatment, each individual apple was rinsed for 30 s under cold

Table 2—Population reduction in Golden Delicious apples inoculated with *Escherichia coli* (ATCC 25922) and decontaminated by static or dynamic sanitizing wash treatment

Treatment	Application method[a]	*E. coli* population reduction[b] (log_{10}CFU/g)
5% H_2O_2 @ 50 °C for 1 min	Static	1.84[d]
	Dynamic	2.81[c]
200 mg/L Cl_2 @ 20 °C for 1 min	Static	1.74[d]
	Dynamic	1.48[d]

[a]Static = immersion without agitation; dynamic = immersion with agitation.
[b]Based on inoculated control *E. coli* population of 5.47 log_{10}CFU/g; enumerated on *E. coli* Petrifilm plates.
[c, d]Means of duplicate trials; means with no letter in common are significantly different at $p < 0.05$ by the LSD test.

Table 3—Population reduction in Golden Delicious Apples inoculated with *E. coli* (ATCC 25922) and decontaminated by targeted abrasion of calyx and stem areas

Treatment[a]	*E. coli* population reduction[b] (log_{10} CFU/g)
Wash, abrade, rinse with H_2O	3.61[d]
Wash, abrade, rinse with 5% H_2O_2	5.28[c]
Wash without abrasion, rinse with H_2O	1.69[e]

[a]Apples washed with 1% APL Kleen® 245 + 5% H_2O_2 @ 50 °C for 1 min; calyx and stem areas abraded with conical grinding tool; rinsed with H_2O or 5% H_2O_2 @ 20 °C for 30 s.
[b]Based on surviving *E. coli* population of treated apples, compared to inoculated control population of 5.78 log_{10}CFU/g; enumerated on *E. coli* Petrifilm plates.
[c, d, e]Means of duplicate trials; means with no letter in common are significantly different at $p < 0.05$ by the LSD test.

tap water to assure removal of residual H_2O_2 on the apple surface. Treated apples and inoculated controls were compared with apples given a "dynamic" wash with 5% H_2O_2 at 60 °C in duplicate trials. The higher treatment temperature was not used with the vacuum treatment to avoid boiling of the H_2O_2 solution and consequent interference with infiltration of the solution into the apple calyx channel. Treated apples and controls were cored and dissected as described above or examined without dissection. Hydrogen peroxide residues in vacuum treated apples were determined with the EM Reflectoquant™ Analysis System (EM Industries, Inc., Gibbsville, N.J., U.S.A.) by cutting apples in half along the stem/calyx axis and applying an EM Quant® peroxide test strip (EM Industries, Inc.) to the exposed calyx channel.

High temperature treatments

Unwaxed Golden Delicious, Granny Smith, Fuji, and Red Delicious apples were immersed in water or 5% H_2O_2 for 30 to 180 s at 60 to 80 °C with agitation to determine their tolerance to high temperature treatment. Treated apples and controls were examined by 2 of the investigators for visual evidence of skin discoloration or for presence of subsurface softening of the apple flesh. Apples were cut in half along the stem/calyx axis so that any subsurface discoloration associated with softening or tissue breakdown could be seen. Apples were periodically examined for delayed development of these or other defects during storage for 7 d at 4 °C. To determine treatment efficacy, duplicate sets of 3 Golden Delicious apples, inoculated with *E. coli* (ATCC 25922), were treated by immersion with agitation in 2 L preheated water or 5% H_2O_2 at 65 or 80 °C for 1 to 3 min in 3-L beakers placed in a 89-L water bath (Precision Circulating Water Bath, Model 270, Precision Scientific Inc., Chicago, Ill., U.S.A.) maintained at 65 or 80 °C. Following treatment, the apples were rinsed with cold tap water in a colander for 1 min and then evaluated for surviving *E. coli*. Treated apples and inoculated controls were compared in triplicate independent trials.

Microbiological methods

Treated apples and controls were weighed, cut into quarters on a sterile cutting board, combined with an equal volume (w/v) of sterile 0.1% peptone water (PW) (Difco) and homogenized for 1 min at medium speed in a sterile 4-L stainless steel Waring blender (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn., U.S.A. To obtain isolated stem and calyx area portions, skin around the periphery of the stem and calyx at a radial distance of about 13 to 15 mm from the stem and calyx axis was 1st removed with a sharp, sterile (flamed) knife to avoid cross contamination. Then the apples were cored with a sterile, stainless steel cork borer, pushed into the fruit from the stem end. Stem and calyx portions were removed from the isolated cores on a sterile cutting board using a sterile knife. Stem and core portions from replicate apples in each sample were separately pooled, weighed, combined with 4 parts PW (w/v), and homogenized in a 1-L glass blender (Waring) for 1 min at medium speed. Blended samples were filtered through a sterile filter bag designed for microbiological examination of particulate suspensions (40 mm pore size; Spiral Biotech, Bethesda, Md., U.S.A.) and diluted as required with sterile PW.

Generic *E. coli* counts were estimated by plating 1 mL aliquots on *E. coli*/coliform count Petrifilm plates (3M Microbiology Products, St. Paul, Minn., U.S.A.) and 0.1 mL aliquots on Trypticase Soy Agar (TSA) (Difco) with a spiral plater (Autoplate 4000, Spiral Biotech). The TSA plates were overlayed after 2 to 3 h incubation at 35 °C with MacConkey agar (MAC) (Difco) to recover injured cells. In the preliminary brushing trials, Brain Heart Infusion Agar (Difco) containing streptomycin sulfate (20 mg/ml; Sigma Chemical Co., St. Louis, Mo., U.S.A.) was used to enumerate *E. coli* ATCC 35695 in the isolated calyx and system portions. TSA/MAC was used to enumerate surviving *E. coli* ATCC 25922 in the undissected apple samples prepared in the follow-up brushing experiments.

Statistical analyses

Population reduction data were analyzed for differences in response to treatments by ANOVA, t-tests and the LSD test to separate means (Miller 1981). All statistical analyses were performed with SAS/STAT software (SAS Institute Inc. 1989).

Results and Discussion

Selection of surrogate

A surrogate for *E. coli* O157:H7 was employed in this study to avoid exposure of laboratory personnel to the human pathogen as a consequence of aerosol production, sprays, and splashes likely to result from some of the experimental decontamination treatments investigated. A recent surrogate screening study carried out in our laboratory, demonstrated similarities between *E. coli* O157:H7 and strains ATCC 25922 and 970152 in growth characteristics on various media, attachment to apples, resistance to H_2O_2, and resistance to heat (Riordan and others 2001). Therefore, these potential surrogates were compared with *E. coli* O157:H7 in representative washing trials with inoculated apples (Table 1). Similar responses provided by O157:H7 and ATCC 25922 to 2-H_2O_2 wash treatments justify use of this surrogate in research on development of these apple decontamination treatments.

Table 4—Population reduction in Golden Delicious Apples inoculated with *E. coli* (ATCC 25922) and decontaminated by washing and targeted brushing with calcinated oyster shell powder

Treatment	*E. coli* population reduction[c] (log_{10} CFU/g)			
	Expt. A	Expt. B	Expt. C	Mean[d]
1% APL Kleen® 245 + 5% H_2O_2 @ 50 °C[a]	1.42 ± 0.05	2.14 ± 0.46	2.19 ± 0.68	1.92[f]
1% APL Kleen® 245 + 5% H_2O_2 @ 50 °C; calcinated oyster shell[b]	2.98 ± 0.12	2.55 ± 0.74	2.69 ± 0.21	2.74[e]

[a]Apples washed with 1% APL Kleen® 245 + 5% H_2O_2 @ 50 °C for 1 min and rinsed with H_2O.
[b]Apples washed with 1% APL Kleen® 245 + 5% H_2O_2 @ 50 °C for 1 min and rinsed with H_2O; then calcinated oyster shell/water paste (1:2) applied to calyx and stem areas followed by brushing with electric toothbrush for 30 s and rinsing with H_2O
[c]Based on inoculated control *E. coli* populations of 4.74, 5.05 and 4.86 log_{10}CFU/g for Expts. A, B and C, respectively; means of duplicate trials for each experiment.
[d]Enumerated on TSA with MacConkey agar overlay.
[e, f]Within the same column, means with no letter in common are significantly different at $p < 0.05$ by the LSD test

Table 5—Population reduction in excised calyx and stem areas of Golden Delicious Apples inoculated with *E. coli* (ATCC 25922) and decontaminated by dynamic washing or vacuum infiltration with 5% H_2O_2, with or without stem removal[a]

Treatment	Stem removal	*E. coli* population reduction[b] (log_{10} CFU/g)	
		Stem tissue[c]	Calyx tissue[c]
5% H_2O_2 at 60 °C (dynamic wash)	No	3.13[d]	2.58[f]
	Yes	2.84[d]	3.18[ef]
5% H_2O_2 vac. infilt. at 45 °C	No	1.46[e]	4.52[de]
	Yes	3.22[d]	4.98[d]

[a]Apples washed with 5% H_2O_2 at 60 °C for 2 min (dynamic wash) or vacuum infiltrated with 5% H_2O_2 at 45 °C and 508 mm Hg for 3 min; apples rinsed with H_2O after treatment.
[b]Based on inoculated control (without stem removal) *E. coli* population of 5.19 and 6.46 log_{10}CFU/g in stem and calyx areas, respectively; enumerated on *E. coli* Petrifilm plates.
[c]Stem and calyx tissues excised aseptically from individual apples, weighed and homogenized for enumeration of surviving *E. coli*.
[d, e] Means of duplicate trials; within columns, means with no letter in common are significantly different at $p<0.05$ by the LSD test.

Table 6—Population reduction in Golden Delicious Apples inoculated with *E. coli* (ATCC 25922) by washing or vacuum infiltrating with 5% H_2O_2 or Cl_2, with or without stem removal

Treatment[a]	*E. coli* population reduction[b] (log_{10} CFU/g)	
	With stems	Without stems
5% H_2O_2 at 60 °C for 2 min	2.26[d]	1.96[de]
5% H_2O_2 vac. infilt. at 45 °C for 3 min	3.12[c]	3.90[c]
200 mg/L Cl_2 at 50 °C for 2 min	1.48[e]	1.52[e]
200 mg/L Cl_2 vac. infilt. at 20 °C for 3 min	1.52[e]	2.15[d]

[a]Apples given dynamic wash or vacuum infiltration at 508 mm Hg for 3 min; then rinsed with H_2O after treatment.
[b]Based on inoculated control (without stem removal) *E. coli* population of 5.10 and 5.05 log_{10}CFU/g for H_2O_2 and Cl_2 trials, respectively; enumerated on *E. coli* Petrifilm plates.
[c, d, e]Means of duplicate trials; within columns, means with no letter in common are significantly different at $p<0.05$ by the LSD test.

Effect of agitation

Our studies (Sapers and others 2000; Riordan and others 2001) and others (Delaquis and others 2000; Fatemi and Knabel 2001) have shown that bacteria may attach to the apple surface in the stem and calyx areas. Because of the possibility that air bubbles entrapped in these areas or fruit-to-fruit contact during washing might limit exposure of attached bacteria to sanitizers, we compared H_2O_2 and Cl_2 wash treatments, applied by complete immersion of fruit with or without agitation, as described. Vigorous agitation of the apples in 5% H_2O_2 at 50 °C, as provided by the dynamic wash procedure, increased the population reduction by 1 log, compared to treatment without agitation (Table 2). However, vigorous agitation during washing did not increase population reductions with the Cl_2 treatment. Population reductions obtained with Cl_2 were similar to those reported by others (Beuchat and others 1998; Wisniewsky and others 2000; Wright and others 2000) and smaller than those obtained with H_2O_2, as we reported (Sapers and others 2000). The improvement in efficacy of H_2O_2 obtained with agitation may have been due to dispersal of oxygen bubbles generated by catalase-catalyzed or spontaneous decomposition of H_2O_2 in the calyx and stem areas, thereby allowing for better contact with bacterial attachment sites. Vigorous agitation of the apples did not appear to cause any bruising or puncturing of the apple skin.

Effect of washing with abrasion

Calyx and stem areas of inoculated apples were washed with 5% H_2O_2 and then abraded with a conical grinding tool to expose bacteria attached in inaccessible sites and disrupt any biofilms in these regions of the apple surface. Population reductions in the calyx and stem areas were 4.48 ± 1.14 and 4.44 ± 1.08 log_{10}CFU/g, respectively, when 6 apples were individually washed with 5% H_2O_2 at 50 °C followed by abrading with the grinding tool and rinsing with 5% H_2O_2. This treatment yielded population reductions of 3.61 and 5.28 log_{10}CFU/g on a whole apple basis when the abraded areas were flushed with water and 5% H_2O_2, respectively (Table 3). Treatment without abrasion resulted in a population reduction of only 1.69 log_{10}CFU/g. While the ability of targeted abrasion to provide large reductions in *E. coli* populations on inoculated apples is apparent, it is not clear whether this treatment could be applied commercially. Weight losses due to removal of stem and calyx tissue were between 2% and 3%. The treatment would require individual apples to be precisely oriented and positioned at high speed between opposing grinding devices with simultaneous flushing to remove contaminated debris. The treatment might be applicable to production of fresh-cut slices but could not be used for fresh market apples due to tissue injury in the stem and calyx areas. Population reductions obtained with apples intended for cider production could not be counted towards the required 5-log reduction, which must be based on treatments applied to the juice (FDA 2001).

An alternative means of decontaminating calyx and stem areas by washing with abrasion was explored using a rotating brush (electric toothbrush) with an abrasive paste. In a preliminary experiment, an experimental calcinated oyster shell product described by Isshiki and Azuma (1995) was applied to inoculated ap-

Table 7—Effect of immersion in heated H_2O and 5% H_2O_2 on quality of apples

Treatment temp. (°C)	Appearance: Quality attribute	H_2O wash	5% H_2O_2 wash
		Golden Delicious	
65	Color	No browning Dark lenticels	No browning Dark Lenticels
	Heat injury[a]	None	None
	Stability[b]	Darker	Darker
80	Color	Browning	Severe browning
	Heat injury	Slight	Slight
	Stability[b]	Darker	No change
		Granny Smith	
65	Color	Slight browning Visible lenticels	No browning Visible lenticels
	Heat injury[a]	None	None
	Stability	No change	No change
80	Color	Olive	Severe browning
	Heat injury[a]	Moderate	Moderate
	Stability	Darker	Darker
		Fuji	
65	Color	No browning	Slight browning
	Heat injury[a]	None	None
	Stability	No change	No change
80	Color	Mod. browning	Severe browning
	Heat injury[a]	Slight	Slight
	Stability[b]	Darker	No change
		Red Delicious	
65	Color	No browning	Slight browning
	Heat injury[a]	None	None
	Stability	No change	Darker
80	Color	Mod. Browning	Severe browning
	Heat injury[a]	Slight	Slight
	Stability[b]	Darker	Darker

[a]Fruit surface less firm and/or dark layer beneath skin.
[b]Change in color or surface firmness after 1 week at 4 °C.

ples with *E. coli* ATCC 35695 (K-12) as the surrogate. Population reductions in the calyx area of 4.4 to 5.2 resulted from the calcinated oyster shell treatment, compared to 2.6 to 3.3, obtained by brushing with water without this abrasive paste. Population reductions in the stem area were smaller and more variable (data not shown). Isshiki and Azuma (1995) reported that the calcinated oyster shell preparation was effective in inhibiting growth of bacteria when added to mashed potatoes and potato salad.

In a follow-up study of brushing treatments with calcinated oyster shell powder as the abrasive material, *E. coli* ATCC 25922 was employed as the surrogate. The inoculated apples were prewashed in 1% APL Kleen® 245 + 5% H_2O_2, followed by application of the brushing treatment to the calyx and stem areas of each individual apple. Population reductions, determined on a whole apple basis, were consistently higher with the combined treatment than with the wash alone, approaching 3 logs with the former treatment (Table 4). This treatment did not result in mechanical injury to the fruit. However, orientation of fruit for treatment is inherently difficult to control, and adaptation for high-speed packing and fresh-cut processing operations may be problematic.

Brushing without an abrasive paste produced inconsistent results, even when the brushed area was simultaneously flushed with 5% H_2O_2, and a wetting agent, 0.2% sodium 2-ethylhexyl sulfate, was added to the flushing solution (data not shown).

Vacuum infiltration

Vacuum infiltration was investigated as a means of improving contact between sanitizing agents and microbial binding sites. When 5% H_2O_2 was applied to inoculated apples at 45 °C under 508 mm Hg vacuum, population reductions in the calyx area were greater than 4 logs, about 2 logs higher than could be obtained by a dynamic wash at 60 °C and atmospheric pressure (Table 5). Removal of the apple stem improved the performance of the vacuum infiltration treatment but not the dynamic wash treatment in the stem area. Presumably, bacterial attachment sites near the base of the stem were made more accessible to the vacuum treatment by stem removal. When population reductions were determined on a whole apple basis, vacuum infiltration of de-stemmed inoculated apples with 5% H_2O_2 at 45 °C yielded a 3.9 $\log_{10}$CFU/g reduction, 2 logs greater than could be obtained at 60 °C and atmospheric pressure (Table 6). In contrast, vacuum infiltration did not appear to improve efficacy of the 200 ppm Cl_2 treatment. Similar results were obtained whether the treatment was applied by submerging the apples in 5% H_2O_2 at atmospheric pressure and then placing them under vacuum, or by placing the apples in a beaker under vacuum and then submerging them in the H_2O_2 solution, added to the beaker through an inlet line to the vacuum oven (data not shown). The vacuum infiltration treatment had little effect on apple quality other than a slight darkening of lenticels in some varieties. H_2O_2 residues in the calyx area decreased to about 5 ppm within 3 h and were not detectable in 18 h.

Vacuum infiltration has been used commercially to infuse sugar, citric acid, and ascorbic acid into apple slices (Hall 1989). Previously, we investigated use of pressure and vacuum infiltration to increase uptake of sodium erythorbate-based browning inhibitors by diced and sliced fresh apples (Sapers and others 1990). Vacuum and pressure infiltration have been used experimentally to increase the calcium content of intact apples as a means of increasing firmness (Poovaiah and Moulton 1982; Abbott and Conway 1989), suppressing decay (Conway and Sams 1983), and reducing bitter pit and low temperature breakdown (Scott and Wills 1979). Vacuum infiltration of H_2O_2 might be applicable to disinfection of apples intended for fresh market or fresh-cut processing.

High temperature treatments

Successful use of heated H_2O_2 solutions to reduce bacterial populations on contaminated apples requires that the apples withstand the required treatment without undergoing discoloration, loss of firmness, or breakdown during storage. Golden Delicious, Granny Smith, Fuji, and Red Delicious apples showed no indications of quality loss or injury at 60 °C other than slight darkening of Golden Delicious lenticels following exposure to 5% H_2O_2 for 180 s (data not shown). At 65 °C, Golden Delicious and Granny Smith apples showed no overall browning but slight darkening of lenticels following exposure to H_2O or 5% H_2O_2 after only 30 s (Table 7). Slight darkening of Golden Delicious occurred during post-treatment storage at 4 °C for 1 wk. Slight browning of Fuji and Red Delicious was detected after 120 s in 5% H_2O_2. No softening of the fruit surface or other defects was observed. It appears that 65 °C represents an upper limit beyond which treatment-induced discoloration becomes conspicuous. At 70 and 80 °C, Golden Delicious, Granny Smith, and Red Delicious all showed moderate browning with H_2O treatment and more severe browning with 5% H_2O_2 treatment, both defects intensifying during post-treatment storage. Fuji tolerated 70 °C during treatment with H_2O and 5% H_2O_2, but samples heated for more than 30 s showed browning during post-treatment storage. At 80 °C, Fuji was similar to the other varieties. All apples heated at 80 °C showed subsurface softening. More comprehensive quality evaluations, carried out using storage conditions typical of commer-

Table 8—Population reduction in Golden Delicious Apples inoculated with *E. coli* (ATCC 25922), immersed in H_2O or 5% H_2O_2 at 65 or 80 °C

		E. coli population reduction[b] (log_{10} CFU/g)	
Treatment[a]	Temp. (°C)	TSA/MAC	Petrifilm
5% H_2O_2	65	2.11[d]	2.61[d]
	80	3.96[c]	3.96[c]
H_2O	65	1.66[d]	2.22[d]
	80	2.16[d]	2.72[d]

[a]Immersed for 2 min with continuous agitation.
[b]Based on inoculated control *E. coli* population of 5.00; enumerated on TSA with MacConkey agar overlay.
[c, d]Means of triplicate trials; means within columns with no letter in common are significantly different at $p < 0.05$ by the LSD test.

cial practice, would be required to demonstrate the absence of long-term degradative changes induced by exposure to H_2O_2 and/or high temperature treatments.

Exposure of inoculated apples to H_2O or 5% H_2O_2 at 65 °C resulted in a 2-log population reduction (Table 8). At 80 °C, the population reduction resulting from exposure to water was the same as at 65 °C, but the population reduction resulting from the peroxide treatment increased to almost 4 logs. These results were obtained by plating on TSA with the MAC overlay, which should have permitted recovery of injured bacterial cells. Generally similar results were obtained by plating on Petrifilm; log reductions obtained with this medium were not significantly higher than those obtained with TSA/MAC, although an apparent trend towards greater log reduction values was seen with the Petrifilm data. This trend suggests that heat or peroxide-induced injury may have affected the accuracy of enumeration with Petrifilm. Consequently, we are using the TSA/MAC procedure to enumerate survivors of all treatments carried out at elevated temperatures. The presence of some survivors, even at 80 °C, demonstrates the inability of a hot water treatment to pasteurize the surface of apples when the targeted bacteria have become well established in protected attachment sites. Similar results were reported by Fleischman and others (2001) with apples inoculated by immersion in a suspension of *E. coli* O157:H7. In that study greater log reductions were obtained with surface-inoculated apples where the attached bacteria were more vulnerable to hot water treatment. Our results indicate that a hot water or H_2O_2 treatment, applied at a time/temperature condition capable of inactivating bacteria in inaccessible sites, would result in excessive heat damage incompatible with product quality. These results support the FDA position that the required 5-log reduction for fresh cider should be based on treatments applied to the juice (FDA 2001). Conceivably, a brief exposure to steam might be capable of large reductions in microbial populations on the surface of apples intended for fresh-cut or fresh market applications without injury to the product because of better heat transfer and utilization of the latent heat of vaporization.

Conclusions

THE EFFICACY OF H_2O_2-BASED SANITIZING TREATMENTS FOR FRESH apples can be improved by increasing contact between the sanitizing solution and inaccessible microbial binding sites on the apple surface. Such improvements can be realized by applying H_2O_2 wash treatments with vigorous agitation, by abrading stem and calyx areas while flushing with the H_2O_2 solution, by targeted brushing with an antimicrobial abrasive paste, by vacuum infiltration of the H_2O_2 solution, and by increasing the treatment application temperature. However, abrasion and high temperature treatments may result in mechanical or thermal injury to the product, which would limit treatment applicability. Further research is required to overcome these limitations and to develop practical means of treatment application so that apples intended for fresh-cut or fresh market applications can be sanitized more effectively.

References

Abbott JA, Conway WS. 1989. Postharvest calcium chloride infiltration affects textural attributes of apples. J Amer Soc Hort Sci 114:932-936.

Annous BA, Sapers GM, Mattrazzo AM, Riordan DCR. 2001. Efficacy of washing with a commercial flat-bed brush washer, using conventional and experimental washing agents, in reducing populations of *Escherichia coli* on artificially inoculated apples. J Food Prot 64:159-163.

Beuchat LR. 1998. Surface Decontamination of Fruits and Vegetables Eaten Raw: a Review. Food Safety Unit, World Health Organization, WHO/FSF/FOS/98.2.

Beuchat LR, Nail BV, Adler BB, Clavero MRS. 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. J Food Prot 61:1305-1311.

Brackett RE. 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. Postharvest Biol Technol 15:305-311.

Conway WS, Sams CE. 1983. Calcium infiltration of Golden Delicious apples and its effect on decay. Phytopathol 73:1068-1071.

Delaquis PJ, Ward SM, Stanich K. 2000. Evaluation of pre-pressing sanitary treatments for the destruction of *Escherichia coli* O157:H7 on apples destined for production of unpasteurized apple juice. Technical Report No. 9901. Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC V0H 1Z0.

Fatemi P, Knabel SJ. 2001. Destruction of E. coli O157:H7 within the internal structure of apples by high pH/temperature differential treatment (abstract). In: IFT Annual Meeting Book of Abstracts; 2001 June 23-27; New Orleans. La. Chicago, Ill.: Institute of Food Technologists. P 149. Abstract 59F-27.

FDA. 2001. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice; final rule. Fed Register 66 (13):6137-6202.

Fleischman GJ, Batore C, Merker R, Keller SE. 2001. Hot water immersion to eliminate *Escherichia coli* O157:H7 on the surface of whole apples: thermal effects and efficacy. J Food Prot 64:451-455.

Hall GC. 1989. Refrigerated, frozen, and dehydrofrozen apples. In: D.L. Downing, editor. Processed apple products. New York: Van Nostrand Reinhold, p 247.

Isshiki K, Azuma K. 1995. Microbial growth suppression in food using calcinated calcium. JARQ 29:269-274.

Miller RG, Jr. 1981. Simultaneous Statistical Inference. New York: Springer-Verlag, p. 90-94.

NACMCF. 1999. Microbiological safety evaluations and recommendations on fresh produce. National Advisory Committee on Microbiological Criteria for Foods. Food Control 10:117-143.

Poovaiah BW, Moulton GA, inventors. Assignee: Washington State University Research Foundation, Inc., Pullman, Wash. May 25, 1992. Vacuum pressure infiltration process for fresh produce. U.S.Patent 4,331,691.

Riordan DCR, Sapers GM, Kwaczak R, and Annous BA. 2001. Unpublished data. USDA-ARS, Eastern Regional Research Center, Wyndmoor, PA.

Sapers GM, Garzarella L, Pilizota V. 1990. Application of browning inhibitors to cut apple and potato by vacuum and pressure infiltration. J Food Sci 55:1049-1053.

Sapers GM, Miller RL, Jantschke M, Mattrazzo AM. 2000. Factors limiting the efficacy of hydrogen peroxide washes for decontamination of apples containing *Escherichia coli*. J Food Sci 65:529-532.

Sapers GM, Miller RL, Mattrazzo AM. 1999. Effectiveness of sanitizing agents in inactivating *Escherichia coli* in Golden Delicious apples. J Food Sci 64:734-737.

SAS Institute Inc. 1989. SAS/STAT Users' Guide, Version 6, Volume 2, 4th ed. Cary, North Carolina.

Scott KJ, Wills RBH. 1979. Effects of vacuum and pressure infiltration of calcium chloride and storage temperature on the incidence of bitter pit and low temperature breakdown of apples. Aust J Agric Res 30:917-928.

Tauxe R, Kruse H, Hedberg C, Potter M, Madden J, Wachsmuth K. 1997. Microbiological hazards and emerging issues associated with produce. A preliminary report to the National Advisory Committee on Microbiologic Criteria for Foods. J Food Prot 60(11):1400-1408.

Wisniewsky MA, Glatz BA, Gleason ML, Reitmeier CA. 2000. Reduction of *Escherichia coli* O157:H7 counts on whole fresh apples by treatment with sanitizers. J Food Prot 63:703-708.

Wright JR, Sumner SS, Hackney CR, Pierson MD, Zoecklein BW. 2000. Reduction of *Escherichia coli* O157:H7 on apples using wash and chemical sanitizer treatments. Dairy, Food and Environ Sanit 20:120-126.

MS20010475 Submitted 8/29/01, Accepted 10/16/01, Received 10/16/01

The authors acknowledge the technical assistance of Matthew R. Evans and Donyel M. Jones at the Eastern Regional Research Center and Dr. John G. Phillips, Consulting Statistician, North Atlantic Area, ARS, for analysis of the data. We also thank Dr. Kenji Isshiki at the National Food Research Institute, Tsukuba, Japan, for providing the calcinated oyster shell powder.

Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Authors are with the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038. Direct inquiries to author Sapers (E-mail:gsapers@arserrc.gov).